FORM PTO-1390 REV. 2/01T — U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	03715 0110
	U S APPLICATION NO (If known, see 37CFR1.5) **10/069405**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/02376	August 25, 2000	August 27, 1999

TITLE OF INVENTION
PROCESS FOR ENCAPSULATING ACTIVE MATERIALS BY COACERVATION OF POLYMERS IN NON-CHOLRINATED ORGANIC SOLVENT

APPLICANT(S) FOR DO/EO/US
Jean-Pierre BENOIT, Joël RICHARD, Elvire FOURNIER, and Sonia LIU

Applicant(s) herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c)(2)).
   - a. ☐ is attached hereto (required only if not communicated by the International Bureau.
   - b. ☒ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed with the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)).
   - a. ☒ is attached hereto.
   - b. ☐ has been previously submitted under 35 U.S.C. 154 (d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3)).
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made, however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A Substitute specification.
16. ☐ A change of power of attorney and/or address letter
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154 (d)(4)
19. ☐ A second copy of the English language translation of the international application 35 U.S.C. 154 (d)(4).
20. ☒ Other items or information:
    - a. ☒ Copy of cover page of International Publication No WO 01/15799
    - b. ☐ Copy of Notification of Missing Requirements.
    - c. ☒ Verification of translation

U.S. APPLICATION NO (If known, see 37CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/069405	PCT/FR00/02376	03715 0110

21. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTOand all claims satisfied provisions of PCT Article 33 (1)-(4) **$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	$890.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$	

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	21	- 20 =	1	x $18.00	$18.00	
Independent Claims	1	-3 =		x $84 00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				+$280.00	$	

TOTAL OF THE ABOVE CALCULATIONS =	$908.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.	$	
SUBTOTAL =	$908 00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	908 00	
Fee for recording the enclosed assignment (37 CFR 1.21 (h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. +	$	
TOTAL FEES ENCLOSED =	$908.00	
	Amount to be refunded:	$
	charged:	$

a. ☒ A check in the amount of $ 908.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-0916. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.
1300 I Street, N.W.
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DATED: February 26, 2002

SIGNATURE

Ernest F. Chapman /25,961

NAME/REGISTRATION NO.

PATENT
Attorney Docket No. 03715.0110
CUSTOMER NUMBER 22,852

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. national phase of PCT/FR00/02376	Group Art Unit: Not assigned
Inventor: Jean-Pierre BENOIT et al.	Examiner: Not assigned
Serial No.: 10/069,405	
Filed: February 26, 2002	

For: PROCESS FOR ENCAPSULATING ACTIVE MATERIALS BY COACERVATION OF POLYMERS IN NON-CHOLRINATED ORGANIC SOLVENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Please add the following new paragraph and new heading on the first page, first line, of the specification, after the title.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is based on and claims the benefit of French application 99/10,854, filed August 27, 1999, and International application PCT/FR00/02376, filed August 25, 2000. The entire disclosure of these applications is relied upon and incorporated by reference herein.

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REMARKS

Entry of this Preliminary Amendment prior to examination is respectfully required. The amendment to the specification adds priority data. No new matter is added by this addition.

The examiner is respectfully requested to consider the above preliminary amendment prior to examination of the application.

If there are any other fees due in connection with the filing of this amendment, please charge the fees to Deposit Account No. 06-0916. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our deposit account.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: May 29, 2002

By: _____
Ernest F. Chapman
Reg. No. 25,961

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR00/02376

VERIFICATION OF A TRANSLATION

I, Susan POTTS BA ACIS

Director to RWS Group plc, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare:

That the translator responsible for the attached translation is knowledgeable in the French language in which the below identified international application was filed, and that, to the best of RWS Group plc knowledge and belief, the English translation of the international application No. PCT/FR00/02376 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: February 11, 2002

Signature of Director :

For and on behalf of RWS Group plc

Post Office Address : Europa House, Marsham Way,
Gerrards Cross, Buckinghamshire,
England.

Process for encapsulating active materials by coacervation of polymers in non-chlorinated organic solvent

The present invention relates to a process for microencapsulating an active principle by coacervation, used especially for preparing sustained-release pharmaceutical forms.

Microencapsulation techniques are conventionally used for separating incompatible chemical substances, for converting liquids into powders, for improving the bioavailability of active principles, for masking the unpleasant taste or odor of certain compounds and for preparing sustained-release pharmaceutical forms.

Sustained-release pharmaceutical forms may be administered subcutaneously or intramuscularly and may be found directly in the blood flow or close to the organ to be treated, and as such biodegradable polymers are often chosen to form part of their composition.

Sustained-release systems based on biodegradable polymers may be administered parentally without removal by surgical operation, since the biodegradable polymers become converted in the body into metabolites that are eliminated via the natural routes. The active principle is released according to kinetics that are modulated by

the diffusion of the active principle and the degradation process of the polymer. Furthermore, the patient's compliance with the treatment is improved since a less frequent administration is involved.

Among the biodegradable polymers frequently used in the encapsulation of active principles are poly(α-hydroxy acids), especially polylactic acids (PLAs) and polylactic acid-glycolic acid (PLAGAs), poly-ε-caprolactone, polyorthoesters, for instance Chronomer® and Alzamer®, polyanhydrides, especially the copolymer of sebacic acid and of (carboxyphenoxy)propane, and biodegradable natural polypeptides and polymers, for instance albumin, bovine serum albumin, collagen and chitosan.

Two main types of microencapsulation technique exist:

- solvent-free techniques, for instance spray-congealing, extrusion (coextrusion/spheronization), gelation, prilling and precipitation of supercritical solutions (RESS), and

- solvent techniques, for instance nebulization, coacervation, emulsion-evaporation, emulsion-extraction, and variants thereof starting with water/oil/water double emulsions.

The prolonged contact of sustained-release pharmaceutical forms with an aqueous medium justifies the use of polymers of more or less hydrophobic nature, which are thus mainly soluble in an organic medium. However, biodegradable polymers based on controlled-release systems are poorly soluble in solvents of low potential toxicity (category 3 solvents according to the ICH standard).

Accordingly, the standard microencapsulation techniques (coacervation and emulsion-evaporation) essentially use chlorinated solvents, for instance dichloromethane (category 2 solvent according to the ICH standard, i.e. solvent to be limited), as solvent for the polymer. However, it is a chlorinated solvent that is known for its neurotoxicity. The permitted residual level of dichloromethane in the finished product is 600 ppm according to standard ICH4.

Whichever microencapsulation technique is used, the microparticles obtained contain residual amounts of solvents. It thus appears to be necessary to develop novel microencapsulation methods that do not involve chlorinated solvents. Two major solutions arise for solving this problem.

One solution for achieving encapsulation without chlorinated solvents is based on methods using no solvent, but certain polymers cannot be used according

to these methods. In addition, the properties of the particles obtained by these methods do not necessarily meet the demands of a long-term treatment.

Another solution consists in replacing chlorinated solvents with non-toxic solvents. The microencapsulation methods which use chlorinated solvents have been widely studied and the variables are known. However, the replacement of chlorinated solvents with nonchlorinated solvents modify the physicochemical interactions between the various components of the formulation. The behavior of biodegradable polymers in the replacement solvents is very different from that in the chlorinated solvents. Thus, poly(L-lactide) and poly(D,L-lactide) are insoluble in ethyl acetate or acetone and no polymer is soluble in ethanol, which is, however, a favored solvent since it is of low toxicity.

The present invention proposes a process of microencapsulation by coacervation that does not use any chlorinated solvent. More specifically, the invention relates to a process of coacervation by addition of nonsolvent. Coacervation by addition of nonsolvent requires the use of three miscible solvents; one of these three solvents is a solvent for the polymer and the other two are nonsolvents for the polymer.

The principle of the coacervation is based on the controlled desolvation of a polymer dissolved in an organic solvent containing an active principle generally in particulate form, induced by addition of a nonsolvent or a polymer-coacervating agent. The solubility of the polymer in the organic solvent is lowered and two immiscible phases form: the coacervate gradually settles out at the surface of the active principle. The addition of a curing agent allows the formation of a continuous polymer film around the active principle.

The active principle particles may be liquid or solid. The active principle may also be initially dissolved in the solvent for the polymer. In this case, it reprecipitates in particulate form when the coacervating agent is added, or can form a homogeneous solid solution in the polymer particles derived from the coacervation.

Study of the polymer/solvent/coacervating agent interactions, for each combination, makes it possible to produce a phase diagram so as to define the ideal polymer/solvent/coacervating agent ratio required for efficient encapsulation. However, it is difficult to predict the encapsulation of an active principle, since the interface properties, in relation with the molecular interactions between the polymer, the solvent and the coacervating agent, change constantly with the

composition of the coacervate (Thomassin C., Merkle H.P., Gander B.A., Physico-chemical parameters governing protein microencapsulation into biodegradable polyester by coacervation, Int. J. Pharm., 1997, 147, 173-186).

The major problem of the coacervation technique is the possible aggregation of particles. In an attempt to solve this, authors have proposed reducing the temperature of the system, essentially in the curing step. The walls are then solid enough to prevent adhesion. Solutions such as the use of chlorofluorocarbons (CFCs) or reduction of the temperature cannot be transposed to the industrial scale. On the other hand, the use of silicone oil is capable of stabilizing the system by virtue of its viscosity (Ruiz J.M., Tissier B., Benoit J.P., Microencapsulation of peptide: a study of the phase separation of poly(D,L-lactic acid-co-glycolic acid)copolymers 50/50 by silicone oil, Int. J. Pharm., 1989, 49, 69-77).

Despite high residual contents of solvents, the coacervation technique remains a technique of choice for the encapsulation of fragile active principles, and especially of water-soluble active principles, in nonaqueous medium.

The choice of the solvent/coacervating agent/curing agent combinations is guided by various criteria:

- the solvent must dissolve the polymer; it is preferable for it not to dissolve the active principle, although the process can still be used with an active principle that is soluble in the solvent for the polymer;

- the coacervating agent must be miscible with the solvent for the polymer. It must not be a solvent for the polymer, otherwise this would amount to a simple transfer of the polymer from the solvent to the coacervating agent. Furthermore, it must not dissolve the active principle, in order to limit the encapsulation losses;

- the curing agent must be partially miscible with the solvent for the polymer, so as to facilitate the extraction. It must not dissolve either the polymer or the active principle, otherwise the encapsulation yield would be greatly reduced.

In the prior art, the coacervation technique uses dichloromethane or chloroform as solvent for the polymer, a silicone oil as coacervating agent and heptane as curing agent.

The present invention relates to a process for microencapsulating an active principle by coacervation, which consists of

- the controlled desolvation or coacervation of a polymer dissolved in an organic solvent containing said active principle, said coacervation being induced by addition of a nonsolvent and being reflected by the deposition of the polymer at the surface of said active principle, and then

- the curing of the polymer deposit by addition of a curing agent, said curing being reflected by the formation of a continuous film coating said active principle,

characterized in that

- the solvent for the polymer is a nonchlorinated organic solvent with a boiling point of between 30°C and 240°C and a relative dielectric permittivity of between 4 and 60, advantageously chosen from ethyl acetate, N-methylpyrrolidone, methyl ethyl ketone, acetic acid and propylene carbonate, and mixtures thereof,

- the nonsolvent is an alcohol or a ketone containing 2 to 5 carbon atoms and preferably 2 or 3 carbon atoms, in particular ethanol (ε=24), 2-propanol (ε=18), 1,2-propanediol (ε between 18 and 24) and glycerol (ε=40), or methyl ethyl ketone (ε=18),

- the curing agent is chosen from water, alcohols containing 1 to 4 carbon atoms, on condition that the curing agent is an alcohol that is different than the nonsolvent, and mixtures thereof.

Although N-methylpyrrolidone is of category 2, like dichloromethane, its limit concentration is markedly higher (4840 ppm as opposed to 600 ppm for dichloromethane).

Advantageously, the nonsolvent and the curing agent are chosen, respectively, from the following pairs: 1,2-propanediol and 2-propanol, glycerol and 1,2-propanediol, glycerol and 2-propanol, 2-propanol and 1,2-propanediol.

According to one preferred embodiment, the polymer is a biodegradable polymer with a weight-average molecular mass (Mw) of between 10,000 and 90,000 g/mol and preferably between 15,000 and 50,000 g/mol, and with a polydispersity index (Ip) of between 1 and 3.5 and preferably between 1.5 and 2.5.

A certain number of other additional characteristics will be indicated below to illustrate various preferred embodiments of the invention.

According to these additional characteristics, the polymer is a lactic acid polymer (PLA) or a copolymer of lactic acid and of glycolic acid (PLAGA).

The polymer is a PLAGA such that Mw is between 15,000 and 20,000 g/mol, preferably equal to 17,500, Ip is between 1 and 2, preferably equal to 1.6, and the percentage of glycolic acid is less than 30%, preferably equal to 25%.

The polymer concentration in the solvent is between 1% and 10% (w/v) and preferably about 4% (w/v).

The nonsolvent/solvent ratio by volume is between 1/2 and 1/1.

The coacervation temperature is less than the glass transition temperature of the polymer, preferably less than or equal to 25°C, preferably less than 4°C and more preferably less than or equal to -4°C.

The nonsolvent is added in successive doses from 200 μl to 1 ml.

The coacervation is performed with stirring, for example with magnetic stirring, at a speed of between 200 and 1000 rpm.

The curing agent also contains a surfactant, the concentration of said surfactant in the curing agent being between 0.5% and 10% (v/v).

The surfactant is a sorbitan ester, for example Tween® 80, or polyvinyl alcohol.

The curing agent/solvent ratio by volume is between 5/1 and 180/1 and preferably between 15/1 and 120/1.

The microspheres are cured with stirring, for example magnetic stirring at a speed of between 500 and 1500 rpm.

The curing temperature is less than or equal to 25°C, preferably less than 4°C and more preferably less than or equal to 0.5°C.

The curing agent is added in several portions and preferably in at least four portions.

The curing lasts between 2 and 4 hours.

The microparticles obtained after the curing operation are filtered through Millipore® system, by centrifugation or through fluted paper.

When the active principle forms a dispersion in the solution for the polymer, the solvent and the

nonsolvent have a viscosity that is high enough to stabilize the active principle.

The particle size of the active principle is between 1 and 50 microns and preferably between 5 μm and 30 μm.

According to one preferred embodiment, the solvent is N-methylpyrrolidone, the nonsolvent is ethanol and the curing agent is water.

According to another preferred embodiment, the solvent is ethyl acetate, the nonsolvent is 2-propanol and the curing agent is water. The polymer is a 75:25 PLAGA such that the Mw is between 15,000 and 20,000 and preferably equal to 17,500, and the Ip is between 1 and 2 and preferably equal to 1.6.

According to a third preferred embodiment, the solvent is acetic acid, the curing agent is water and the polymer is a 50:50 PLAGA.

In the context of the present invention, when the active principle is insoluble in the solvent for the polymer, either a suspension or an emulsion is prepared.

To prepare the suspension, the active principle is ground using a mortar and then placed in suspension in the solvent. The suspension may be homogenized by

magnetic stirring: the coacervation is then also performed with magnetic stirring. The suspension may also be homogenized with mechanical stirring, at variable speed (impeller stirrer, Heidolph RGL500, Prolabo, Paris, France) or using an Ultra-Turrax® T25 mixer (Prolabo, Paris, France). In these two latter cases, the coacervation is then performed with mechanical stirring.

Dispersing the active principle in the polymer solution may also be conventionally performed with ultrasound agitation.

When the active principle is water-soluble, to prepare the emulsion, the active principle is dissolved in water and the water/solvent emulsion of the polymer is then prepared with mechanical stirring. The coacervation then takes place with mechanical stirring.

When the active principle is soluble in the solvent for the polymer, the coacervation is performed with mechanical stirring.

In the context of the present invention, the polymer is a biodegradable polymer frequently used in the encapsulation of active principles, preferably a PLA or a PLAGA, more preferably a PLAGA with a weight-average molar mass (Mw) of between 10,000 and 90,000, a number-average molar mass (Mn) of between 4000 and 40,000, a

polydispersity index (Ip) of between 1 and 3.5, and for which the proportion of glycolide is between 10 and 60%. Polymers with a high weight-average molar mass of greater than or equal to 15,000 g/mol will be preferred, since they allow the manufacturing yield to be increased by increasing the volume of the coacervate phase. Polymers with a low polydispersity index ($Ip \leq 2.6$) will also be preferred, since the fractions of low molecular weight remain in solution and bring about a reduction in yield, or cause the aggregation of the microparticles by sticking to their surface.

The PLAGA is, for example, Resomer® RG 502 (Boehringer Ingelheim, Mw = 14,300 g/mol), Mn 6900 g/mol, Ip 2.5, 50% glycolide), Resomer® RG 756 (Mw 89,800 g/mol, Mn = 35,200 g/mol, Ip 2.6, 25% glycolide), Resomer® RG 858 (Mw = 87,000 g/mol, Mn 22,000 g/mol, Ip 3.9, 15% glycolide), Phusiline supplied by Phusis (Mw = 17,500 g/mol), Mn 10,940 g/mol, Ip 1.6, 25% glycolide).

The polymer concentration in the solvent must be sufficient to increase the viscosity of the medium, which makes it possible to stabilize the dispersed coacervate droplets and to limit their aggregation, on the one hand, and to reduce the formation of small-sized microparticles, on the other hand.

The polymer concentration is preferably between 1 and 10% (w/v) and more preferably equal to about 4% (w/v).

The viscosities of the solvent and the nonsolvent must be sufficient to stabilize the coacervate droplets.

The volume of nonsolvent to be added is defined so as to bring the system into the window of stability and to obtain a stable coacervate. However, the volume of nonsolvent also depends on the concentration of the active principle crystals in suspension in the organic solution of polymer.

The addition of an excess of nonsolvent makes it possible to accelerate the curing of the wall of the microparticles, to prevent their aggregation and to improve the extraction of the solvent.

The rate of addition of the nonsolvent is low enough to prevent the formation of a large number of excessively small microparticles, i.e. between 1 and 2 μm in size. In addition, the slower the phase separation, the more uniform the particle size distribution of the microparticles and the smoother the surface of the microparticles. The addition of nonsolvent preferably takes place gradually in doses from 200 μl to 1 ml, waiting for at least one minute between each dose.

Reducing the stirring speed during the coacervation step increases the size of the coacervate droplets and then of the final microparticles. However, below a limit speed, which varies depending on the systems, the kinetics of deposition of the coacervate are too slow and/or the coacervate droplets are too large and not sufficiently stable. A mechanical or magnetic stirring of between 200 rpm and 1000 rpm often gives good results.

The temperature is the essential parameter of the coacervation; it must be less than the glass transition temperature of the polymer. The lower the temperature, the more viscous the medium and the less the microparticles are prone to aggregate.

The ideal curing agent should not dissolve either the active principle or the polymer. It should readily extract the solvent for the polymer. The curing agent used is water optionally supplemented with surfactant or an alcohol. Water advantageously allows the solvent for the polymer to be readily extracted. It also has the advantage of being cheap and of not requiring reprocessing of the effluents. However, water is not the ideal curing agent in the case of water-soluble active principles, since any prolonged contact is responsible for diffusion of the active principle, which is reflected by a low degree of encapsulation.

When the encapsulated active principle is hydrophilic during the curing, it is rapidly dissolved by the water, which penetrates into the microparticles, and can back-diffuse out of the particles. By working at low temperature, the diffusion phenomena are reduced, and thus the losses of active principle toward the aqueous phase are reduced, and the encapsulation yield is improved.

Other possibilities may be envisaged for reducing the diffusion of the active principle; for instance saturation of the outer phase with an electrolyte or the active principle itself, if it is cheap, and combining water with another solvent that has strong affinity for the solvent for the polymer, so as to extract microspheres. Thus, the volume of water used is reduced and the contact with water is attenuated.

The surfactant or the alcohol make it possible to limit the self-aggregation of the microparticles so as to form a homogeneous dispersion. They have been selected on the basis of their harmlessness. The surfactants are chosen from those commonly used in formulations for injection such as polyoxyethylenated sorbitan esters, for instance Tween® 80 and Tween® 20 (hydrophilic surfactants).

Montanox® 80 (polyoxyethylenated sorbitan monooleate) is a hydrophilic emulsifier that may be used in the composition of an emulsion of oil/water type.

Montane® 80 (sorbitan oleate) is its equivalent in the lipophilic surfactant range. Solutol® HS 15 (polyethylene glycol 660 hydroxystearate) is a nonionic surfactant of hydrophilic nature used in injectable solutions.

Synperonic® PE/F 68 (Poloxamer 188) is a bock copolymer of polyoxyethylene and of polyoxypropylene.

Finally, polyvinyl alcohol has been used in two different grades: Mowiol® 4/88 and Rhodoviol® 4/125.

The volume of curing agent is based on a compromise. It must be sufficient to rapidly remove the solvent for the microspheres, but it will have to limit the diffusion of the active principle out of the microspheres. The volume is defined from the solubility criteria of the solvent in the outer phase, such that the final concentration of the solvent in the curing agent is less than the saturation concentration of solvent in the outer phase. By rapidly removing the solvent, the diffusion of the active principle is prevented by the formation of a polymer barrier. Furthermore, the microspheres will be cured, thus preventing aggregation.

The increase in the volume of the curing agent, which takes place gradually by additions at regular intervals, makes it possible to better extract the solvent for the microparticles.

The ratio of the volume of the curing agent to the volume of the solvent is between 5/1 and 180/1 and preferably between 15/1 and 120/1.

Combinations using acetic acid or N-methylpyrrolidone as solvent for the polymer require little curing agent to give solid microspheres, such that the ratio of the volume of the curing agent to the volume of the solvent is advantageously about 5/1 in this case.

The method for drying microparticles depends on the rigidity of the microspheres, the size and the volumes to be treated.

The tendency of the microparticles to aggregate after drying depends on their degree of hydration and on the residual amounts of solvent.

If drying in the open air is insufficient, placing under vacuum and/or increasing the temperature allow it to be completed, on condition that the microparticles can withstand the vacuum. However, it is necessary to

ensure that the drying rate and temperature are not too high, to prevent aggregation of the microparticles.

In the case of combinations using ethyl acetate as solvent for the polymer, the problem arises of choosing the method for separating out the microspheres. Specifically, the microspheres are still engorged with solvent after stirring for one hour in the curing agent. Filtration on a Millipore® system, through a filter of porosity 0.5 μm, gives rise to a solid cake that is difficult to redisperse. Furthermore, the filter is then quickly clogged with the microspheres, which are still deformable. Separation by centrifugation on an aliquot of the solution did not give the hoped for result. In this case also, the microspheres aggregate and form a pellet that cannot be redispersed, even at a relatively low centrifugation speed. Another filtration technique using a fluted filter paper is found to be a solution. This process has the advantage of a large filtration surface and is performed at atmospheric pressure. However, the microspheres of smallest size are also prone to be adsorbed into the pores and gradually clog the filter. It is also difficult to recover all of the microspheres. As regards drying, this is carried out with a stream of compressed air and the microspheres are then left in ambient air.

According to one embodiment of the invention, the solvent for the polymer is ethyl acetate, the nonsolvent is 2-propanol and the curing agent is a water/surfactant mixture, optionally a water/surfactant/alcohol mixture.

In this embodiment, the polymer is preferably a 75:25 PLAGA. The polymer concentration is between 1 and 5% (w/v) and preferably equal to about 4% (w/v). The coacervation is conducted at ambient temperature, preferably at a temperature below 4°C, more preferably less than or equal to -4°C, with mechanical stirring, preferably at 300 rpm.

The surfactant concentration is between 1 and 10% (v/v). The surfactant is Tween® 80. When an alcohol is combined with water, the surfactant concentration is set at between 1 and 10% approximately and the alcohol concentration is set at between 2.5 and 5% approximately. The alcohol is advantageously 2-propanol or 1,2-propanediol. The aqueous solution of curing agent is added in at least four portions. The curing operation lasts for at least 2 hours 30 minutes and for not more than 4 hours, and is carried out at ambient temperature, preferably at a temperature below 4°C, more preferably at 0.5°C, with mechanical stirring (500 rpm).

The nonsolvent/solvent ratio by volume is equal to 1/2.

The curing agent/solvent ratio by volume is equal to 120/1.

The lower the curing temperature, the shorter the curing operation will be. Thus, when the temperature is below 4°C, a duration of 4 hours is sufficient. The duration is lowered to 2 hours 30 minutes when the temperature is 0.5°C.

According to another embodiment, the solvent is N-methylpyrrolidone, the nonsolvent is ethanol and the curing agent is a water/surfactant mixture. The polymer concentration is between 4 and 10% (w/v). The coacervation and curing operation are performed at ambient temperature, with magnetic stirring. The surfactant concentration is between 0.5 and 10% (v/v). The curing time is between 2 and 4 hours. The curing agent/solvent ratio by volume is equal to 40/1. The polymer is preferably Resomer RG® 502 or Resomer RG® 756.

The present invention is illustrated with the examples which follow, without limiting its scope.

<u>Example 1:</u> Assessment of the polymer/solvent/coacervating agent/curing agent combinations.

In this study, the poly(α-hydroxy acids) were assessed. Three copolymers of lactic acid and glycolic acid, in which the proportions of L- and D-lactides and glycolides are variable, were used. These are the following polymers, supplied by Boehringer Ingelheim:

- Resomer® RG 502 (Mw = 14,300 g/mol, Mn = 6900 g/mol), which comprises 25% L-lactide, 25% D-lactide and 50% glycolide,

- Resomer® RG 756 (Mw = 89,800 g/mol, Mn = 35,200 g/mol), which contains 37.5% L-lactide, 37.5% D-lactide and 25% glycolide,

- Resomer® RG 858 (Mw = 87,000 g/mol, Mn = 22,000 g/mol), which has 42.5% L-lactide, 42.5% D-lactide and 15% glycolide.

In view of the respective solvents for the polymers, the least toxic solvents were selected. They belong to solvents of category 2 or 3, defined by the classification of the ICH guidelines.

The miscibility of the solvent/nonsolvent pairs for the three polymers studied was then determined. Similarly,

the nonsolvents for the polymers were chosen for their low toxicity.

Screening of the solvent/nonsolvent/curing agent combinations is performed in scintillation flasks, on small volumes of organic solutions of polymers at 1 or 4% (for N-methylpyrrolidone and 50:50 PLAGA exclusively) (m/v), 5 ml of organic solution of polymer are placed in a scintillation flask. The coacervating agent is then added until a cloudiness which persists on stirring, which is characteristic of the formation of coacervate, is obtained. The coacervate is observed at this step by optical microscopy. Next, 1 ml of this mixture is poured into 10 ml of an aqueous solution of surfactant. The presence or absence of microspheres is observed by optical microscopy.

a) Coacervation tests with 50:50 PLAGA (Resomer® RG 502)

The solvents in which 50:50 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylacetamide, dimethylformamide, ethyl lactate, N-methylpyrrolidone and propylene carbonate. 50:50 PLAGA is insoluble in toluene, 2-propanol, glycerol, dioctyl adipate, 1,2-propanediol, xylene, diethyl carbonate and methyl ethyl ketone.

The combinations for which the formation of a coacervate and of microspheres is observed are the following:

- ethyl acetate/2-propanol/water + Tween® 80,
- acetic acid/1,2-propanediol or 2-propanol/water + Tween® 80,
- N-methylpyrrolidone/ethanol/water + Tween® 80 or 2-propanol,
- N-methylpyrrolidone/methyl ethyl ketone or 2-propanol/water + Tween® 80,
- propylene carbonate/2-propanol/water optionally with Tween® 80, ethanol or NaCl.

Acetic acid may be used as solvent for the polymer and to give rise to the formation of coacervate and of correctly individualized microspheres, on condition that water is used as curing agent. Effectively, droplets of coacervate form in the acetic acid/2-propanol combination, but in the presence of methyl ethyl ketone or 1,2-propanediol as curing agents, the coacervate droplets do not conserve their spherical shape and lumps of polymers are formed.

The ethyl acetate/2-propanol/water + Tween® 80 combination gives good results, as does the N-methylpyrrolidone/ethanol/water + Tween® 80 combination. This study was completed by the investigation of curing agents. 1,2-Propanediol and 2-propanol are found to be good candidates. However, a reduced capacity for extracting the solvent may be noted with 1,2-propanediol than in the presence of 2-propanol.

Propylene carbonate is an advantageous solvent due to its low toxicity and also its partial solubility in water. The propylene carbonate/2-propanol combination makes it possible to dispense with water by virtue of the possibility of replacing the outer phase with a solvent or, at least, of limiting the diffusion of the active principle, by adding an electrolyte in the outer phase or by mixing water and a solvent.

b) Coacervation tests with 75:25 PLAGA (Resomer® RG 756)

The solvents in which 75:25 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylacetamide, dimethylformamide, diethyl ether, methyl ethyl ketone and N-methylpyrrolidone.

75:25 PLAGA is insoluble in toluene, 2-propanol, glycerol, 1,2-propanediol, propylene carbonate, dioctyl adipate and triethyl citrate.

The two solvents that give the best results are acetic acid and ethyl acetate. The studies were thus focused on assessing acetic acid/glycerol and acetic acid/1,2-propanediol combinations and also ethyl acetate/2-propanol and ethyl acetate/1,2-propanediol pairs.

The combinations for which the formation of individualized microspheres is observed are:

- ethyl acetate/1,2-propanediol/water + Tween® 80 or 2-propanol,

- acetic acid/glycerol or 1,2-propanediol/water + Tween® 80.

c) Coacervation tests with 85:15 PLAGA (Resomer® RG 858)

The solvents in which 85:15 PLAGA is soluble are ethyl acetate, acetone, acetonitrile, acetic acid, dimethylformamide, ethanolamine, ethylenediamine, methyl ethyl ketone, N-methylpyrrolidone, toluene and triethyl citrate. 85:15 PLAGA is insoluble in 2-propanol, glycerol, 1,2-propanediol, dioctyl adipate and xylene.

The combinations for which the formation of coacervate and microspheres is observed are:

- ethyl acetate/2-propanol or 1,2-propanediol/water + Tween® 80,
- ethyl acetate/2-propanol/1,2-propanediol,
- ethyl acetate/1,2-propanediol/2-propanol,
- acetic acid/glycerol (water + Tween® 80) or 1,2-propanediol or 2-propanol,
- acetic acid or methyl ethyl ketone/1,2-propanediol/water + Tween® 80,
- acetic acid/2-propanol/1,2-propanediol,
- N-methylpyrrolidone or methyl ethyl ketone/2-propanol/water + Tween® 80,
- methyl ethyl ketone/1,2-propanediol/2-propanol.

Example 2: Production of microspheres not containing active principle, by varying the coacervation parameters

In a first stage, the operating conditions are established for the preparation of microspheres without active principle, so as to produce particles of the desired size. The effect of various factors, for

instance the volume of coacervating agent added, the volume of curing agent, the type and speed of stirring, and the method for collecting the microspheres, is studied.

The polymer is dissolved in 50 ml of organic solvent (Beaker No. 1) to give a 1% solution (w/v). The polymer concentration is brought to 4% (w/v) when the solvent is N-methylpyrrolidone. With stirring, the coacervating agent is added until a stable and visible coacervate is obtained. The mixture is then poured into a solution of curing agent supplemented with a surfactant (Beaker No. 2), with stirring. The microspheres are then recovered by filtration. The combinations tested are those selected in example 1.

a) With 50:50 PLAGA (Resomer® RG 502)

The various protocols corresponding to each of the combinations selected are summarized in table 1.

TABLE 1

Procedures defined for the formulations of microspheres not containing active principle, based on 50:50 PLAGA								
Combinations Solvent/nonsolvent (with water + surfactant as curing agent	Volume of nonsolvent	Stirring Beaker 1	Speed Beaker 1	Stirring Beaker 2	Speed Beaker 2	Volume of curing agent	Filtration method	Size of the micro-spheres
Ethyl acetate/2-propanol	9 ml	Magnetic	500 rpm	Magnetic	700 rpm	750 ml	Fluted filter	40 μm
Acetic acid/1,2-propanediol	16 ml	Ultrasound		Magnetic	1100 rpm	250 ml	Fluted filter	24 μm
Acetic acid/2-propanol	16 ml	Ultrasound		Magnetic	1400 rpm	250 ml	Fluted filter	67 μm
N-Methylpyrrolidone/ethanol	31 ml	Mechanical	200 rpm	Magnetic	1000 rpm	1000 ml	Centrifuga-tion	31 μm
N-Methylpyrrolidone/2-propanol	40 ml	Magnetic	300 rpm	Magnetic	1000 rpm	500 ml	Fluted filter	70 μm

b) Application to 75:25 PLAGA (Resomer® 756)

Microspheres are obtained under the conditions described in table 2 with ethyl acetate as solvent. Three stirring possibilities appear for the ethyl acetate/1,2-propanediol/water + Montanox® 80 or Tween® 80 combination: agitation by ultrasound, mechanical paddle stirring and magnetic stirring. There are no appreciable differences in the morphology or size of the microspheres that can justify the choice of one of these methods.

TABLE 2

Procedures defined for the formulations of microspheres not containing active principle, based on 75:25 PLAGA								
Combinations Solvent/nonsolvent (with water + surfactant as curing agent	Volume of nonsolvent	Stirring Beaker 1	Speed Beaker 1	Stirring Beaker 2	Speed Beaker 2	Volume of curing agent	Filtration method	Size of the micro-spheres
Ethyl acetate/1,2-propanediol	15 ml	Ultrasound		Magnetic	1100 rpm	750 ml	Fluted filter	20 μm
Ethyl acetate/1,2-propanediol	15 ml	Mechanical	400 rpm	Magnetic	1100 rpm	750 ml	Fluted filter	25 μm
Ethyl acetate/2-propanol	16 ml	Magnetic	900 rpm	Magnetic	1100 rpm	800 ml	Fluted filter	32 μm

Example 3: Production of microspheres containing 5-fluorouracil (5-FU) as active principle

The tests are performed with the combinations of example 1 a) and b), which led to the observation of a coacervate.

a) Effect of the active principle on the coacervation

The active principle at low concentration (5% by weight) forms with the polymer solution a homogeneous dispersion. The stability of the dispersion depends on the solvents; thus, N-methylpyrrolidone, which is a viscous solvent, leads to a better stability of the dispersion and the losses of antimitotic agent by decantation or adsorption onto the walls of the beakers are reduced.

The use of 1,2-propanediol as coacervating agent further stabilizes the system by increasing the viscosity of the medium.

b) Production yield

This ranges from 15 to 100% depending on the combinations. The combinations using ethyl acetate as solvent for the polymer give the best yields, close to 100%.

c) Yield and degree of encapsulation

When the theoretical contents of active principle are increased, the encapsulation yield is very low.

Changing to ultrasound makes it possible to individualize the active principle particles. When low concentrations are used, the dispersion is homogeneous and encapsulation yields of 70% are achieved.

Example 4: Study of the N-methylpyrrolidone/ethanol/water + surfactant combination

Among the N-methylpyrrolidone-soluble polymers tested, the following will be selected:

- Resomer® RG 502 (Boehringer Ingelheim), which is a 50:50 D,L-PLAGA,
- Resomer® RG 756 (Boehringer Ingelheim) and Phusiline(Saint-Ismier), which are 75:25 D,L-PLAGAs.

The initial formulation of the microparticles uses an organic solution of polymer at 4 or 10% (w/v) for a volume of solvent of 5 ml. The coacervation is performed with magnetic stirring. The nonsolvent for the polymer is added using a micropipette, milliliter by milliliter. As soon as cloudiness appears in the solution, a sample is taken and observed by optical

microscopy. One minute after the appearance of the cloudiness, the coacervation medium is poured dropwise into 200 ml of curing agent (water + Tween® 80 or PVA). The curing of the microparticles is performed with mechanical stirring. A further observation of the microparticles by optical microscopy is carried out during the curing. The microparticles are then filtered under vacuum, or at atmospheric pressure on filter paper should it be impossible to filter under vacuum (virtually immediate clogging of the filter or excessively long filtration time). Finally, the microparticles are observed just after filtration and after lyophilization.

The microparticles obtained with the NMP/ethanol/water + surfactant combination are, by optical microscopy, spherical, smooth and uniform, whatever the stage of the formulation and whatever the type of polymer.

For all the batches prepared, the filtration after curing was performed on filter paper and at atmospheric pressure. The size of the microparticles is between 30 and 50 μm. Numerous microparticles about 5 μm in diameter are also observed.

- **Effect of the type of polymer and its concentration**

The microparticles obtained all have the same appearance by optical microscopy.

The best yields are obtained with Resomer® RG 502 and Resomer® RG 756 under the following conditions (table 3).

TABLE 3

Type of polymer	Polymer concentration (w/v)	Type of curing agent	Concentration of the surfactant in the curing agent (v/v)	Curing time	Temperature during the coacervation	Temperature during the curing	Manufacturing yield
RG 502	10%	PVA	0.5%	3 h 30	Ambient	Ambient	23.8%
RG 502	10%	Tween® 80	1%	3 h 30	Ambient	< 4°C	35.9%
RG 756	4%	Tween® 80	1%	2 h	Ambient	Ambient	49.0%
RG 756	4%	Tween® 80	5%	2 h	Ambient	Ambient	38.6%

- **Effect of the nature of the curing agent and of the curing time**

The type of curing agent (water, water + Tween® 80, or water + PVA) for the same polymer 50:50 PLAGA (RG 502) and at ambient temperature, has no influence either on the filtration or on the yield for the manufacture of the microparticles, which remains between 17.5 and 23.8%.

The curing time, for the same polymer 50:50 PLAGA (RG 502) makes it possible to increase the manufacturing yield by about 10%, at ambient temperature.

- **Effect of the temperature**

a) During curing

Curing at a temperature below 4°C made it possible to double the manufacturing yield of the microparticles, for 50:50 PLAGA (RG 502): 35.9 as opposed to 17.5%.

b) During coacervation

The manipulations performed entirely at a temperature below 4°C have a maximum manufacturing yield of 10.5%.

The N-methylpyrrolidone/ethanol/water + surfactant combination leads to the formation of microparticles of a satisfactory appearance by optical microscopy.

Example 5: Microspheres containing progesterone or budesonide

Progesterone and budesonide are hydrophobic active principles that are soluble in ethyl acetate.

The ethyl acetate/2-propanol/water + Tween® 80 combination is used, and 75:25 D,L-PLAGA (Phusis, Saint-Ismier) is used as polymer.

The manufacturing yields are all greater than 90%.

The results obtained are collated in table 4.

TABLE 4

Batch	Polymer Concentration (w/v) and mass AP	Coacervation			Curing						Manufacturing yield	Particle size (μm)
		Method for adding the nonsolvent	Temperature	Stirring	Type	Conc. surfactant	Volume	Temperature	Time	Stirring		
1	4% progesterone 28.5 mg (solution)	200/200 μl 1 min between each addition	-4°C	Mechanical 300 rpm	Tween® 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) +30 min	Mechanical 500 rpm	96.7%	28.0 ± 12.5
2	4% budesonide 30.1 mg (solution)	200/200 μl 1 . min between each addition	-4°C	Mechanical 300 rpm	Tween® 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) +30 min	Mechanical 500 rpm	>99.9%	25.3 ± 9.2

Batch	Polymer Concentration (w/v) and mass AP	Coacervation			Curing						Manufacturing yield	Particle size (µm)
		Method for adding the nonsolvent	Temperature	Stirring	Type	Conc. surfactant	Volume	Temperature	Time	Stirring		
3	4% budesonide 30.8 mg (solution)	200/200 µl 1 min between each addition	-4°C	Mechanical 300 rpm	Tween® 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) +30 min	Mechanical 500 rpm	>99.9%	20.8 ± 8.7
4	4% budesonide 29.8 mg (solution)	200/200 µl 1 min between each addition	-4°C	Mechanical 300 rpm	Tween® 80	10%	300 ml +150 ml +150 ml (filtr.) +300 ml	0.5°C	1 h +30 min + 1 h (filt.) +30 min	Mechanical 500 rpm	97.6%	22.5 ± 9.4

* Filtration under vacuum, Nylon filter

The yields and degrees of encapsulation of the active principles are given in the table below and are calculated as follows:

- Ttheo. = [weight of active principle/(weight of polymer + weight of active principle)] × 100,

- Texp. = [weight of active principle/weight of dry microparticles] × 100.

They make it possible to calculate the encapsulation yield Y:

- Y = [Texp./Ttheo.] × 100

Yields and degrees of encapsulation of progesterone and budesonide with 75:25 PLAGA (Phusis)

Batch	Theoretical degree	Experimental degree	Encapsulation yield	Degree of residual moisture
1 (progesterone)	12.4%	8.2%	66.1%	11.9%
2 (progesterone)	13.1%	9.5%	72.5%	-
3 (budesonide)	13.3%	8.0%	60.1%	-
4 (budesonide)	13.0%	8.8%	67.7%	16.4%

The ethyl acetate/2-propanol/water + surfactant combination leads to the formation of microparticles with a satisfactory appearance by optical microscopy. On the other hand, the microparticles obtained with

this solvent/nonsolvent pair are more fragile and are dried in the open air, thus at ambient temperature and at atmospheric pressure, so as not to be irreversibly aggregated. The manufacturing yields are close to 100%. The aggregation is the main problem encountered with this solvent/nonsolvent/curing agent combination. It may be reduced by modifying the various parameters of the formulation. The temperature at which the coacervation is carried out is specially an essential factor: when lowered to -4°C, it makes it possible to set the polymer and to rigidify the microparticles, which thus become individualized and no longer stick together.

CLAIMS

1. A process for microencapsulating an active principle by coacervation, which consists of

- the controlled desolvation or coacervation of a polymer dissolved in an organic solvent containing said active principle, said coacervation being induced by addition of a nonsolvent and being reflected by the deposition of the polymer at the surface of the active principle, and then

- the curing of the polymer deposit by addition of a curing agent, said curing being reflected by the formation of a continuous film coating said active principle,

characterized in that

- the solvent for the polymer is a nonchlorinated organic solvent with a boiling point of between 30°C and 240°C and a relative dielectric permittivity of between 4 and 60, advantageously chosen from ethyl acetate, N-methylpyrrolidone, methyl ethyl ketone, acetic acid and propylene carbonate, and mixtures thereof,

- the nonsolvent is an alcohol or a ketone containing 2 to 5 carbon atoms and preferably 2 or 3 carbon atoms, in particular ethanol (ε=24), 2-propanol (ε=18), 1,2-propanediol (ε between 18 and 24) and glycerol (ε=40), or methyl ethyl ketone (ε=18),

- the curing agent is chosen from water, alcohols containing 1 to 4 carbon atoms, on condition that the curing agent is an alcohol that is different than the nonsolvent, and mixtures thereof.

2. The process as claimed in claim 1, characterized in that the nonsolvent and the curing agent are chosen, respectively, from the following pairs: 1,2-propanediol and 2-propanol, glycerol and 1,2-propanediol, glycerol and 2-propanol, 2-propanol and 1,2-propanediol.

3. The process as claimed in claims 1 and 2, characterized in that the polymer is a biodegradable polymer with a weight-average molecular mass (Mw) of between 10,000 and 90,000 g/mol, preferably between 15,000 and 50,000 g/mol, and with a polydispersity index (Ip) of between 1 and 3.5 and preferably between 1.5 and 2.5.

4. The process as claimed in claim 3, characterized in that the polymer is a lactic acid polymer (PLA) or a polymer of lactic acid and of glycolic acid (PLAGA).

5. The process as claimed in claim 4, characterized in that the polymer is a PLAGA such that Mw is between 15,000 and 25,000, preferably equal to 17,500, Ip is between 1 and 2, and preferably equal to 1.6, and the percentage of glycolic acid is less than 30%, preferably equal to 25%.

6. The process as claimed in one of claims 1 to 5, characterized in that the polymer concentration in the solvent is between 1 and 10% (w/v) and preferably about 4% (w/v).

7. The process as claimed in one of the preceding claims, characterized in that the nonsolvent/-solvent ratio by volume is between 1/2 and 1/1.

8. The process as claimed in one of the preceding claims, characterized in that the coacervation temperature is less than the glass transition temperature of the polymer, preferably less than or less than or equal to 25°C, preferably less than 4°C and more preferably equal to -4°C.

9. The process as claimed in one of the preceding claims, characterized in that the curing agent also contains a surfactant, the concentration of said surfactant in the curing agent being between 0.1 and 10% (v/v).

10. The process as claimed in one of the preceding claims, characterized in that the surfactant is a sorbitan ester, for example Tween® 80 or polyvinyl alcohol.

11. The process as claimed in one of the preceding claims, characterized in that the curing agent/solvent ratio by volume is between 5/1 and 180/1 and preferably between 15/1 and 120/1.

12. The process as claimed in one of the preceding claims, characterized in that the microspheres are cured with stirring at a speed of between 500 and 1500 rpm.

13. The process as claimed in one of the preceding claims, characterized in that the curing temperature is less than or equal to 25°C, preferably less than 4°C and more preferably less than or equal to 0.5°C.

14. The process as claimed in one of the preceding claims, characterized in that when the active

principle forms a dispersion in the polymer solution, the solvent and the nonsolvent have a viscosity that is high enough to stabilize the active principle.

15. The process as claimed in one of the preceding claims, characterized in that the active principle is dispersed by ultrasound to form a dispersion in the polymer solution, and the coacervation is performed with gentle stirring, preferably of magnetic or mechanical type.

16. The process as claimed in one of the preceding claims, characterized in that the particle size of the active principle is between 1 and 50 micrometers and preferably between 5 µm and 30 µm.

17. The process as claimed in one of the preceding claims, characterized in that the solvent is N-methylpyrrolidone, the nonsolvent is ethanol and the curing agent is water.

18. The process as claimed in one of claims 1 to 16, characterized in that the solvent is ethyl acetate.

19. The process as claimed in claim 18, characterized in that the solvent is ethyl acetate, the

nonsolvent is 2-propanol and the curing agent is water.

20. The process as claimed in claim 18 or 19, characterized in that the polymer is a 75:25 PLAGA such that the Mw is between 15,000 and 20,000 and preferably equal to 17,500, and the Ip is between 1 and 2 and preferably equal to 1.6.

21. The process as claimed in one of claims 1 to 16, characterized in that the solvent is acetic acid, the curing agent is water and the polymer is a 50:50 PLAGA.

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(54) Titre: PROCEDE D'ENCAPSULATION DE MATIERES ACTIVES PAR COACERVATION DE POLYMERES EN SOLVANT ORGANIQUE NON-CHLORE

(57) Abstract: The invention concerns a method for microencapsulation of an active principle by coacervation which consists in controlled desolvation or coacervation of a polymer dissolved in an organic solvent containing said active principle, said coacervation being induced by adding a non-solvent causing the polymer to be deposited at the surface of the active principle, and by hardening the polymer deposit by adding a hardening agent, said hardening being leading to the formation of a continuous film coating the active principle. The invention is characterised in that the solvent is selected among ethyl acetate, N-methylpyrrolidone, methylethylacetone and acetic acid. The non-solvent is advantageously an alcohol comprising two to five carbon atoms and the hardening agent being for instance selected among water, alcohols comprising four carbon atoms and mixtures thereof.

(57) Abrégé: La présente invention concerne un procédé de microencapsulation d'un principe actif par coacervation qui consiste en la désolvatation ménagée ou coacervation d'un polymère dissous dans un solvant organique contenant ledit principe actif, ladite coacervation étant induite par addition d'un non-solvant et se traduisant par le dépôt du polymère à la surface du principe actif, et en le durcissement du dépôt de polymère par ajout d'un agent durcisseur, ledit durcissement se traduisant par la formation d'un film continu enrobant le principe actif, caractérisé en ce que le solvant est choisi parmi l'acétate d'éthyle, la N-méthylpyrrolidone, la méthyléthylcétone et l'acide acétique. Le non-solvant est avantageusement un alcool comprenant deux à 5 atomes de carbone et l'agent durcisseur est par exemple choisi parmi l'eau, les alcools comprenant un à 4 atomes de carbone et leurs mélanges.

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: PROCESS FOR ENCAPSULATING ACTIVE MATERIALS BY COACERVATION OF POLYMERS IN NON-CHLORINATED ORGANIC SOLVENT the specification of which ☐ is attached and/or ☒ was filed on AUGUST 25, 2000 as ~~United States Application Serial No.~~ _____ or PCT International Application No. PCT/FR00/02376 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States, listed below and have also identified below, any foreign application(s) for patent or inventor's certificate, or any PCT International application(s) having a filing date before that of the application(s) of which priority is claimed:

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C.
FRANCE	99/10854	27 AUGUST 1999	☒ YES ☐ NO
			☐ YES ☐ NO

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Number	Date of Filing

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application(s) and the national or PCT International filing date of this application:

Application Number	Date of Filing	Status (Patented, Pending, Abandoned)
PCT/FR00/02376	25 AUGUST 2000	pending

I hereby appoint the following attorney and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W. Edmondson, Reg. No. 33,871; Michael R. McGurk, Reg. No. 32,045; Joann M. Neth, Reg. No. 36,363; Gerson S. Panitch, Reg. No. 33,751; Cheri M. Taylor, Reg. No. 33,216; Charles E. Van Horn, Reg. No. 40,266; Linda A. Wadler, Reg. No. 33,218; Jeffrey A. Berkowitz, Reg. No. 36,743; Michael R. Kelly, Reg. No. 33,924; James B. Monroe, Reg. No. 33,971; Doris Johnson Hines, Reg. No. 34,629; Allen R. Jensen, Reg. No. 28,224; Lori Ann Johnson, Reg. No. 34,498; and David A. Manspeizer, Reg. No. 37,540 and _____. Please address all correspondence to FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P. 1300 I Street, N.W., Washington, D.C. 20005, Telephone No. (202) 408-4000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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